Growth, Water Relations, and Nutritive Value of Pasture Species Mixtures under Moisture Stress

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ABSTRACT

Pasture productivity under harsh environments can be increased by planting more drought-resistant species or by increasing species diversity. This research was conducted under two large (10.2 × 26.8 m) rainout shelters combined with a drip irrigation system to provide deficit, normal, and excessive moisture conditions. A two-species mixture containing the relatively drought-tolerant species, orchardgrass (*Dactylis glomerata* L.) and red clover (*Trifolium pretense* L.) and two five-species mixtures were compared with a mixture containing the drought-sensitive species, white clover (*Trifolium repens* L.) and Kentucky bluegrass (*Poa pratensis* L.), which are the predominant species in northeastern USA pastures. Plots were clipped from mid-May to early October in 2000 and 2001 on a schedule that mimicked management-intensive grazing practices. The five-species mixture containing chicory (*Cichorium intybus* L.), orchardgrass, Kentucky bluegrass, perennial ryegrass (*Lolium perenne* L.), and white clover had the greatest dry matter yield at all moisture levels. Yield in that mixture increased 89% in the dry, 61% in the normal, and 43% (by weight) in the wet treatments compared with the white clover/Kentucky bluegrass mixture. Increased yield was primarily due to the robust growth of chicory which dominated the mixture, accounting for 71% of harvested biomass by the fall of 2001. In addition, white clover growing in the mixture with chicory had improved leaf water relations and greater relative growth rates than white clover growing in the two-species mixture. Including the functional attribute of a deep-rooted forb appeared to be more important than species richness, per se, in improving forage yield.

A PRIMARY GOAL of grazingland managers in the northeastern USA is to improve forage production during summer months when heat and moisture stress limit growth of cool-season forages. Although temperate pastures in the northeastern USA typically contain about 30 plant species (Tracy and Sanderson, 2000), mixtures usually are dominated by Kentucky bluegrass and white clover with infrequent, transient species accounting for most of the species richness. Both dominant species are sensitive to water deficits and elevated temperatures, providing only limited forage during summer months. White clover is particularly intolerant of drought because of its shallow root system and inability to effectively control transpiration (Hart, 1987), which leads to early leaf wilting and senescence. While Kentucky bluegrass can survive severe drought, it does so by going dormant during the dry period (Fergus and Buckner, 1973).

One option to improve summer performance is to plant species with greater drought resistance such as orchardgrass, tall fescue (*Festuca arundinacea* Schreb.), or chicory. Orchardgrass and tall fescue are well-adapted to relatively dry conditions and can survive drought better than many cool-season forage grasses (Burns and Chamblee, 1979; Thomas, 1986; Christie and McElroy, 1994). Chicory is increasingly being considered as a potential forage for temperate regions because of its improved herbage growth when production of other cool-season forages lags and because of its high nutritive value (Belesky et al., 1999, 2000).

Increasing plant species diversity has also been proposed as a means of increasing the productivity and stability of grazinglands facing drought stress (Ruz-Jerez et al., 1991; Daly et al., 1996; Caldeira et al., 2001). Greater plant diversity can improve primary productivity by increasing total resource use (niche differentiation) (Tilman, 1999; Loreau and Hector, 2001) or through positive interactions among neighboring plants (Bertness, 1998). However, some have suggested that the observed increase in productivity with increasing diversity simply results from the increased probability of including the most productive species from the pool of availably species (the sampling effect) (Wardle, 1999).

The purpose of this study was to evaluate alternatives to the Kentucky bluegrass/white clover mixture that dominates northeastern USA pastures to improve forage production under summer moisture conditions ranging from excessively wet to excessively dry. Species mixtures included a drought resistant grass/legume mixture (orchardgrass/red clover) and two five-species mixtures composed of a variety of resistant and susceptible grasses, legumes, and forbs. Although the experiment was not specifically designed to test the effect of increased species diversity on yield, we hypothesized that the five-species mixtures would have higher yields under stressful moisture conditions than the simple grass/legume mixtures.

MATERIALS AND METHODS

Experiments were conducted under movable rainout shelters at the Russell E. Larson Experimental Farm at Rock Springs, PA, on a Murrill silt loam soil (fine-loamy, mixed, semiactive, mesic Typic Hapludult). Soil tests before planting in 1999 indicated that P and K levels were in the optimum-to-high range. Repeated tests in the fall of 2000 indicated that P had decreased from the high-to-midoptimum range while K was below optimum. On 23 April 2001, 34 kg ha^{-1} P and 185 kg ha^{-1} K were applied to all plots. Nitrogen was supplied by the legume component of each mixture. Two 10.2- by 26.8-m rainout shelters are located at the site. These moveable shelters were covered with heavy-duty plastic each spring and

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Abbreviations: CP, crude protein; IVTD, in vitro true digestibility; LWP, leaf water potential; NDF, neutral detergent fiber.

were automatically triggered by rainfall to cover the plots, excluding all natural precipitation during the growing season. Rain gauges placed within the shelters verified that no measurable precipitation was received by the plots while the shelters were operational. The shelters automatically opened following rain storms, exposing experimental plots to ambient radiation and temperature conditions whenever it was not raining.

Experimental plots (1.2 × 1.2 m) were sown within each shelter on 4 August 1999 and irrigated during late summer and early fall, as needed to ensure seedling establishment. Four mixtures were included in the study as follows: (A) Kentucky bluegrass/'Will' white clover, (B) 'Pennlate' orchardgrass/red clover, (C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/'Puna' chicory, and (D) Kentucky bluegrass/perennial ryegrass/'Barcel' tall fescue/red clover/'Tonic' narrow-leaf plantain (*Plantago lanceolata* L.). The five-species mixtures were comprised of three functional groups including grasses (three species that differed in seasonal productivity and responses to moisture stress), a legume, and a deep-rooted forb. Each plot was hand sown at a rate of 1500 seeds per plot with 750 seeds per species in the two-species (simple) mixtures and 300 seeds per species in the five-species (complex) mixtures. Plots were hand weeded during the fall of 1999 and early spring of 2000 to remove seedlings of nonsown species. Following the spring 2000 weeding, no attempt was made to control nonsown plants.

In 2000, all plots were mowed to a 5-cm stubble height on 4 April and again on 9 May to remove standing dead material and early-season growth. Rain was excluded from the plots from 22 May to 4 October 2000. In 2001, plots were mowed on 19 April and rain was excluded from 14 May to 26 September. Rainout shelters were turned off and the plots exposed to ambient weather conditions between 4 October 2000 and 13 May 2001 and after 26 September 2001. While the rainout shelters were in operation, water was applied weekly to each plot through a drip irrigation system with four emitters per plot spaced 60 cm apart. Irrigation treatments were based on long-term May through September weather records for State College, PA. Water was applied to match the average of the 10 wettest (28 mm wk^{-1}), 10 median (21 mm wk^{-1}), and 10 driest (13 mm wk^{-1}) summers for the 92 yr for which precipitation records were available. Volumetric soil moisture content was determined on the day before irrigation water was applied with a Troxler neutron gage (Troxler Electronic Laboratories, Inc., Research Triangle Park, NC). Soil moisture data were collected at the 30- and 60-cm depths.

Plots were clipped by hand on a schedule that was designed to mimic a grazing schedule that would be used by producers using management-intensive rotational grazing. Thus, plots were clipped when plants reached an appropriate height for the species included in each mixture rather than on a predetermined schedule. The first harvest used for biomass determinations occurred on 23 May 2000 and 15 May 2001 and the final harvest on 3 October 2000 and 25 September 2001. About 18% of harvested biomass in 2000 and 25% in 2001 was produced before the rainout shelters were activated and irrigation treatments imposed. Because Mixture A contained low-growing species, it was harvested when plants reached an average height of 15 cm and was cut to stubble heights of either 3 (low) or 6 cm (high). Mixtures B through D were cut to stubble heights of 6 (low) or 10 cm (high) when plants reached a height of 25 cm. Under these cutting regimes, three to seven harvests were taken each year depending on mixture, water application, and stubble height (Table 1). To avoid edge effects, a 0.1-m^2 section (0.2 × 0.5 m) was cut from the center of each plot for dry matter, forage quality, and species composition determinations. The remainder of the plot was then cut to the desired stubble height.

Plots harvested in May, July, and September of each year were hand separated into individual planted species, dead material, and nonplanted species for determination of species composition. All other harvests were dried and weighed without separation. Forage from the May, July, and September harvests was ground to pass a 2-mm screen. Crude protein (CP), neutral detergent fiber (NDF), and in vitro true digestibility (IVTD) were estimated by the Pennsylvania State University Crop Quality Laboratory using near infrared reflectance spectroscopy. Calibration equations were developed from a subset of the experimental material.

Growth rates were calculated for whole plots and for individual species in May, July, and September by dividing the amount of dry biomass collected at a given harvest by the number of days since the last harvest. This assumed that stubble height remained constant across harvests so that all harvested biomass had been produced since the previous cutting. Relative growth rates for individual species were then calculated with the growth rate from the May 2000 harvest as a baseline to adjust for differences in the number of sown seeds per species and for differences in initial establishment. Relative growth rates were expressed as a proportion of the growth rate in May 2000.

Leaf water potential (LWP) and transpiration rates were measured for white clover in both years and for red clover and orchardgrass in 2001 with a pressure chamber (Model 600, PMS Instrument Co., Corvallis, OR) and a steady state porometer (LI-1600, LI-COR Biosciences, Lincoln, NE), respectively, at 3-wk intervals from May to October. For the pressure chamber readings, three leaf samples per plot were collected under shade, placed in humidified zippered bags protected from the sun, and transported to a shaded location

Table 1. Number of harvests per year for forage mixtures cut on a schedule to mimic management-intensive grazing practices. Plots were cut when canopy height reached 15 cm (Mixture A) or 25 cm (Mixtures B–D). Stubble heights were: Mixture A, high 6 cm, low 3 cm; Mixtures B–D, high 10 cm, low 6 cm. Mixtures included the following species: (A) Kentucky bluegrass/white clover; (B) orchardgrass/red clover; (C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/chicory; and (D) Kentucky bluegrass/perennial ryegrass/tall fescue/red clover/narrow-leaf plantain.

	No. of harvests											
	Dry		Normal		Wet		Dry		Normal		Wet	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
	Mixture A						Mixture B					
2000	4	4	5	5	6	6	5	4	6	5	6	5
2001	4	4	5	5	6	6	5	5	6	6	5	6
	Mixture C						Mixture D					
2000	4	4	4	4	5	4	4	3	4	4	4	4
2001	7	7	7	6	7	5	4	4	5	5	5	5

where readings were taken within 20 min. Transpiration readings were taken nondestructively on one leaf per plot. All measurements were made between 1200 and 1330 h EST.

On 21 May 2002, 5-cm-diam. soil cores were collected from the center of each plot to a depth of 90 cm and divided into sections at depths of 0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm. Roots were washed free of soil and oven dried for biomass determinations. Dried samples were ashed in a muffle furnace at 550°C to remove soil contaminants and root weight reported on an ash-free basis. At the same time root cores were collected, aboveground material was also harvested and separated by species as described for the May, July, and September harvests in 2000 and 2001.

Forage yield and nutritive value data and individual species relative growth rates were analyzed as a randomized complete block split-plot design with four replications. Whole-plots within each block consisted of irrigation treatments with species mixture and stubble height treatments as subplots. There were two blocks per rainout shelter. Relative growth rate data were square root transformed before analysis to ensure a normal distribution of the data. The LWP, transpiration rate, and soil water content data were analyzed in a split-plot-in-time design. Soil water content was measured in only two of the four replications.

RESULTS

Climatic Conditions

The rainout shelters and irrigation systems were in operation for 20 wk each year. During that time, the wet treatment received 560 mm of irrigation water, the normal treatment 420 mm, and the dry treatment 260 mm. Mean daily air temperatures during the experiment were identical in 2000 and 2001 (18.0°C), although daytime highs in 2001 averaged 0.9°C higher and nighttime lows 0.9°C lower than in 2000. There were 14 d in 2001 when the high was >30°C, compared with only 4 in 2000. Maximum temperature was 31.1°C in 2000 and 33.9°C, in 2001. Mean air temperature both years was lower than the long-term average for May to September of 18.6°C.

The dry treatment reduced soil moisture ($P = 0.05$) at the 30-cm depth compared with the normal and wet treatments, which did not differ from each other (Fig. 1). Soil moisture content at 30 cm initially decreased under all water treatments following activation of the rainout shelters each spring, reaching minimum values on 12 July 2000 and 25 July 2001. Plant canopies then appeared to adjust water use to match water availability so that soil water content remained fairly stable the remainder of the summer. During September 2000, soil moisture content at 30 cm increased in all irrigation treatments as water consumption became less than supply. Soil moisture at the 60 cm depth remained near field capacity throughout the experiment regardless of irrigation treatment or mixture identity (data not shown). By the end of the experiment, Mixture C had the greatest amount of water remaining in the upper 30 cm of the soil profile in the dry treatment (0.283 m^3 m^{-3}), while Mixtures A, B, and D were significantly lower at 0.261, 0.267, and 0.261 m^3 m^{-3}, respectively ($P < 0.05$).

Species Composition

Mixture A was initially dominated by white clover (86% of harvested biomass), but by the end of the experiment white clover and Kentucky bluegrass biomass were nearly equal (Table 2). Biomass in Mixture B was more evenly distributed among the two sown species throughout the experiment, averaging 56% for orchardgrass and 35% for red clover. However, the proportion of red clover in the mixture tended to decrease as moisture stress increased.

Mixture C was initially dominated by perennial rye-

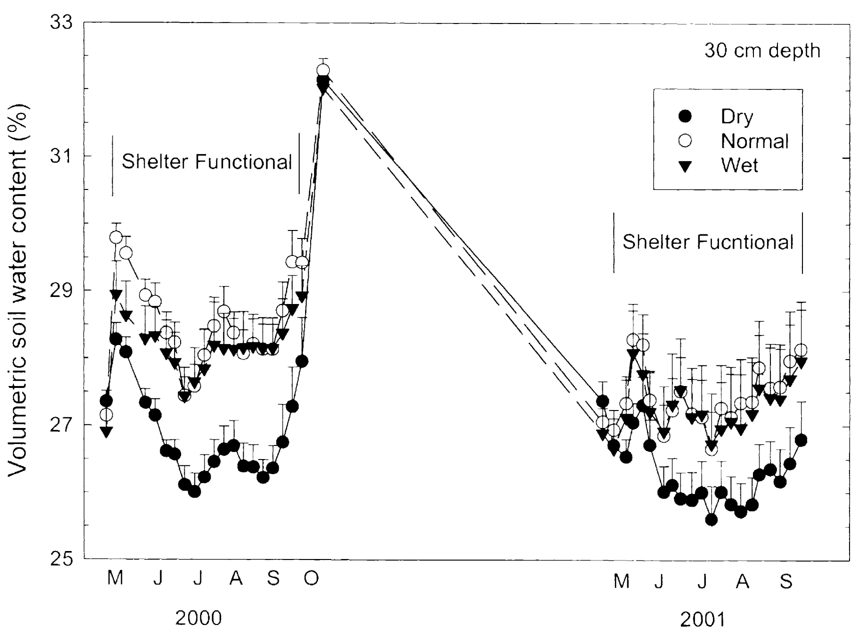

Fig. 1. Effect of irrigation treatment on volumetric soil water content at 30 cm below the soil surface. Rainout shelters excluded rainfall from mid-May through early October each year and 560 mm (wet), 420 mm (normal), or 260 mm (dry) water was applied with a drip irrigation system. Data are averaged across species mixtures. Each data point represents the mean of 8 observations.

Table 2. Botanical composition for four pasture mixtures containing either two or five planted species. Data are averaged across irrigation treatment and stubble height. Species include: Kentucky bluegrass (BG), chicory (CH), orchardgrass (OG), perennial ryegrass (PR), plantain (PT), red clover (RC), tall fescue (TF), white clover (WC), other nonplanted species (OT), and dead material from all species (DD). Species that contributed 10% or more to harvested biomass in the five-species mixtures are shown in italic font.

	Percentage of harvested biomass						
	2000			2001			2002
Species	May	July	Sep	May	Jul	Sep	May
			Mixture A				
BG	3	8	20	34	13	26	44
WC	86	66	51	49	72	43	27
OT	8	8	19	16	9	17	26
DD	3	17	10	T†	6	14	3
			Mixture B				
OG	54	58	61	73	43	48	70
RC	43	35	26	25	49	34	23
OT	1	T	1	2	1	5	5
DD	2	7	12	1	7	13	3
			Mixture C				
BG	0	0	1	2	1	T	3
CH	*31*	*52*	*62*	*46*	*71*	*71*	*49*
OG	*12*	*12*	*12*	*27*	9	9	*31*
PR	*51*	*14*	9	8	T	1	4
WC	5	*17*	8	*11*	9	2	3
OT	1	1	3	5	7	9	8
DD	T	6	5	T	4	7	1
			Mixture D				
BG	T	T	2	5	1	1	3
PR	*59*	*18*	*11*	*21*	1	2	7
PT	1	7	*10*	T	1	1	T
RC	*35*	*54*	*47*	*50*	*66*	*50*	*38*
TF	3	*14*	*20*	*22*	*12*	*35*	*49*
OT	1	T	T	1	*11*	1	2
DD	1	7	10	T	8	11	2

† Trace.

grass (57%) and chicory (31%). However, the proportion of perennial ryegrass quickly decreased and that species had almost disappeared from the mixture by the summer of 2001. Chicory increased throughout the experiment, accounting for 71% of harvested biomass at the July and September 2001 harvests. Mixture D was also initially dominated by perennial ryegrass (59%) along with red clover (35%). As in Mixture C, the perennial ryegrass had almost disappeared from Mixture D by the end of the experiment. Red clover increased across time, averaging 55% of harvested biomass in 2001. Tall fescue also increased during the course of the experiment, from 3% in May 2000 to 35% in September 2001. At every harvest, each five-species mixture was dominated by two of the species in the mixture, although the identity of the dominant species changed with time. The two most abundant species contributed between 72 and 96% of live aboveground biomass at any given harvest.

Encroachment of nonplanted species was minimal in Mixtures B through D, averaging <5% of harvested biomass (Table 1). In Mixture A, however, nonplanted species averaged 14 and 17% of harvested biomass in the wet and normal treatments, but only 8% in the dry treatment. By the May 2002 harvest, biomass of nonplanted species was equal to that of white clover in

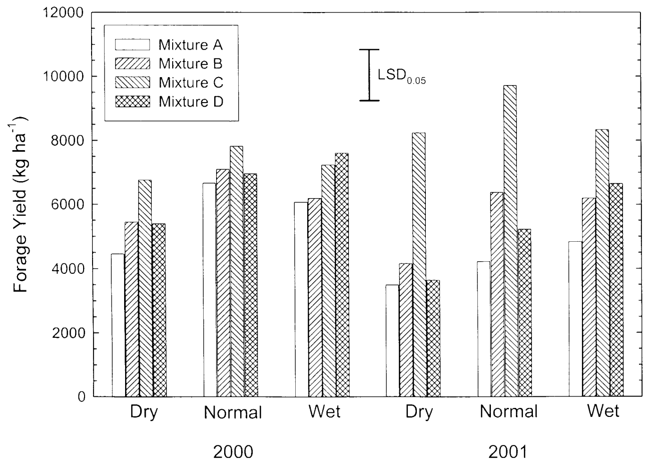

Fig. 2. Total forage yield from 9 May to 4 October 2000 and 19 April to 25 September 2001 for four pasture mixtures. Mixtures included the following species: A) Kentucky bluegrass/white clover; B) orchardgrass/red clover; C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/chicory; and D) Kentucky bluegrass/perennial ryegrass/tall fescue/red clover/narrow-leaf plantain. Water was applied to match the average amount received during the 10 wettest (28 mm wk^{-1}), 10 median (21 mm wk^{-1}), and 10 driest (13 mm wk^{-1}) summers for the 92 years for which precipitation records were available for State College, PA. Data are averaged across stubble heights which had little effect on yield. Each bar represents the mean of 8 observations.

Mixture A. The percentage of dead material increased from 4 and 5% in the wet and normal treatments, respectively, to 10% in the dry treatment ($P < 0.05$). The dead component of the dry treatment was least for Mixture C (4%), intermediate for mixtures D and B (8 and 10%, respectively), and greatest for Mixture A (16%) ($P < 0.01$ for mixture × water interaction).

Forage Yield

There were significant year × mixture, mixture × water, and mixture × stubble × water effects on forage yield. Averaged across years and mixtures, dry matter yield in the wet and normal treatments were not significantly different from each other (6640 and 6760 kg ha^{-1}, respectively), but the dry treatment reduced yield by 22% to 5200 kg ha^{-1} ($P < 0.01$). When averaged across years and moisture treatments, forage yield was lowest in Mixture A, increased by 19% in Mixtures B and D, and increased by 62% in Mixture C ($P < 0.01$). In the dry treatment, only Mixture C had significantly greater yield than Mixture A (89% increase). In the normal treatment both Mixtures B and C had greater yields than Mixture A (24 and 61% increases, respectively), while in the wet treatment Mixtures C and D had greater yields (43 and 23% increases, respectively). Forages yields were significantly lower in 2001 compared with 2000 in Mixtures A and D ($P < 0.01$) and were lower, but not significantly so, in Mixture B as well (Fig. 2). In contrast, yield of Mixture C was 20% greater in 2001 compared with 2000 ($P < 0.01$). Improved yield in Mixture C in 2001 was consistent across all moisture treatments as was the reduced yield in the other mixtures.

There were few differences in May growth rates

Table 3. Species mixture and water treatment effects on growth rates for plots harvested during May, July, and September. Mixtures included the following species: (A) Kentucky bluegrass/white clover, (B) orchardgrass/red clover, (C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/chicory, and (D) Kentucky bluegrass/perennial ryegrass/tall fescue/red clover/narrow-leaf plantain. Plots watered at rates of 13 (dry), 21 (normal), or 28 (wet) mm wk^{-1}.

		Growth rate (dry weight)		
Mixture	Water	May	July	September
		Kg ha^{-1} d^{-1}		
A	Dry	61.2	19.8	14.2
	Normal	52.7	39.3	30.8
	Wet	47.3	39.7	28.9
B	Dry	53.4	28.9	17.0
	Normal	64.1	41.1	33.3
	Wet	52.0	47.0	30.5
C	Dry	62.3	46.7	43.5
	Normal	65.6	59.5	65.0
	Wet	65.5	49.1	57.0
D	Dry	56.5	27.0	18.8
	Normal	55.8	44.9	34.3
	Wet	71.7	53.7	41.9
LSD (0.05)		14.2	10.3	9.6

among mixtures or among moisture treatments (Table 3). Mixture D was the only mixture with significant differences among water treatments, with the wet treatment having a greater growth rate than the normal or dry treatments. The wet treatment in Mixture D also had a greater growth rate than the wet treatment in Mixture A. Otherwise, growth rates in May were the same for all other mixtures and water treatments. With the exception of Mixture D in May and Mixture C in July, there were no significant differences in growth rates between the normal and wet treatments. Growth rate in Mixture C decreased in July in the wet compared with the normal treatment.

The effect of drought on plant growth rate became more severe as the drought continued from summer into early autumn. When averaged across mixtures, the dry treatment reduced growth rate compared with the normal treatment by 33% in July and 43% in September. Drought had the least effect on Mixture C, reducing growth rate by 22 and 33% in July and September, respectively. Mixture A was the most susceptible to drought with growth rate being reduced by 50% in July and 54% in September.

Stubble height had a significant effect on forage yield only for Mixture C (data not shown), where yields in the dry and normal treatments were 36 and 22% greater in the short compared with the tall stubble ($P < 0.01$), while yield was 18% lower in the wet treatment at the shorter stubble height ($P < 0.10$). In general, stubble height had little effect on any of the yield or forage quality parameters examined.

Four species, Kentucky bluegrass, white clover, red clover, and orchardgrass were included in both simple and complex mixtures, providing an opportunity to evaluate the effects of mixture complexity on growth rates of individual species in response to moisture availability. Because growth rate is highly dependent on initial biomass, and because species composition differed depending on mixture complexity and moisture treatment, growth rate data for individual species were normalized,

Table 4. Effect of mixture complexity (simple vs. complex) on July and September relative growth rates for three individual species. Growth rates (g g^{-1} d^{-1}) were calculated for each harvest, then July and September rates were expressed relative to the growth rate in May 2000. Data were square root transformed for data analysis, then means were back transformed for presentation. Mean separation statistics (LSD) were calculated for transformed data. Data are averaged across stubble height and years.

	Percentage of May 2000 growth rate			
	July		September	
Main Effect	Simple	Complex	Simple	Complex
Water				
Dry	35ef†	102ab	17f	45de
Normal	50de	137a	22f	83bc
Wet	69cd	110ab	29ef	81bc
Species‡				
OG	42c	58c	26de	53c
RC	47cd	190a	16e	108b
WC	62c	119b	26de	50c

† For each main effect, means within rows and columns followed by the same letter are not significantly different at $P = 0.05$.
‡ OG, orchardgrass; RC, red clover; WC, white clover.

with May 2000 growth rates for each species by mixture by water treatment combination serving as a baseline (growth rate = 1.00). July and September growth rates were then compared with the May 2000 baseline with data averaged across years. Kentucky bluegrass was not included in the analysis because no Kentucky bluegrass was found in the complex mixtures in May 2000, making it impossible to establish a baseline. In the simple mixtures, relative growth rates for white clover, red clover, and orchardgrass decreased in July compared with May, then decreased again in September (Table 4). Those same species growing in complex mixtures were able to maintain growth in July at equal or greater rates compared with growth during May. Relative growth rates in the complex mixture were greater than in the simple mixture at all moisture levels and for all species with the exception of orchardgrass in July.

Leaf Water Relations

Drought stress reduced LWP in all three species compared with the normal and wet treatments, which did not differ from each other (Table 5). Mixture complexity also affected white clover LWP with the normal and dry treatments having lower water potentials in the simple compared with the complex mixture. Mixture complexity had no effect on red clover or orchardgrass water potential. Neither mixture complexity nor water stress had a strong, predictable effect on orchardgrass transpiration (Table 5). Red clover transpiration was reduced in the dry compared with the normal and wet treatments, which did not differ from each other, whereas mixture complexity had no effect on red clover transpiration. Conversely, white clover transpiration was greater in the complex mixture than in the simple mixture in the normal and dry treatments while there was no difference in white clover transpiration between mixtures in the wet treatment. Stomatal conductance results were similar to transpiration results for all three species (data not shown).

Table 5. Leaf water relations for white clover (WC), red clover (RC), and orchardgrass (OG) growing in two-species (simple) and five-species (complex) mixtures. Values within a given row followed by the same letter are not significantly different at $P = 0.05$. Data for WC are from 2000 and 2001 while data for RC and OG are for 2001 only.

	Simple mixture			Complex mixture			
	Dry	Normal	Wet	Dry	Normal	Wet	LSD (0.05)
	Leaf Water Potential, MPa						
WC	−1.80c	−1.59b	−1.51a	−1.64b	−1.52a	−1.52a	0.07
RC	−1.72b	−1.57a	−1.60a	−1.76b	−1.57a	−1.56a	0.10
OG	−1.87bc	−1.73a	−1.81ab	−1.94c	−1.79ab	−1.82ab	0.11
	Transpiration Rate, $\mu g\ cm^{-2}\ s^{-1}$						
WC	3.60c	4.30bc	4.64ab	5.12ab	5.34a	5.50a	0.92
RC	5.18bc	5.85ab	6.20ab	4.58c	5.94ab	6.47a	1.06
OG	3.00ab	2.41b	4.02a	3.71a	3.30ab	3.68a	1.24

Nutritive Value

Mixture A consistently had greater CP concentration than the other mixtures (Table 6), regardless of time of year, water treatment, or stubble height (average of 220 g kg^{-1} for Mixture A vs. 182 to 184 g kg^{-1} for the other three mixtures, $P < 0.01$). Protein concentration of Mixtures B to D tended to vary from each other by <20 g kg^{-1}, while CP in Mixture A was generally 20 to 60 g kg^{-1} greater than the next highest mixture. The drought treatment reduced CP compared with the normal and wet treatments in July ($P < 0.01$), but water treatment had no effect on protein levels in May or September. In 2000, average protein content decreased from 207 g kg^{-1} in May to 173 and 172 g kg^{-1} in July and September, respectively ($P < 0.01$). In 2001, CP averaged 200 g kg^{-1} and did not change significantly from May to September.

Mixture A had the lowest NDF concentration (330 g kg^{-1}), followed by Mixture C (374 g kg^{-1}), Mixture D (413 g kg^{-1}), and Mixture B (457 g kg^{-1}). All mixtures were significantly different from each other (Table 6). There was also a significant mixture × water interaction for NDF. In Mixtures B and D, NDF increased with increasing water stress, in Mixture A, NDF was greatest under normal water application, whereas NDF of Mixture C was not affected by moisture treatment. Stubble height had no effect on NDF.

In vitro true digestibility values were high, averaging 867 g kg^{-1}. Highest IVTD values were around 890 g kg^{-1} for Mixtures A and C, whereas Mixture D had the lowest IVTD, averaging 844 g kg^{-1} (Table 6). Moisture stress caused a reduction in IVTD for Mixtures A, B, and D ($P < 0.01$), but not for Mixture C, which had significantly higher IVTD than all other mixtures in the dry treatment. Stubble height had no effect on IVTD.

Root Biomass

Plot size was too small to permit routine coring for root biomass determination during the experiment. Therefore, root data were collected during the spring of 2002 to give a general sense of root distribution below the various mixtures. Mixture D had the greatest total root biomass and B the least (Table 7). Averaged across mixtures, 72% of total root biomass was located in the top 15 cm of the soil profile with 4% located between 60 and 90 cm. Mixture A had the shallowest root system with 85% of its biomass in the top 15 cm and <1% between 60 and 90 cm. Conversely, Mixture C had the deepest root system with 52% of the biomass in the top 15 cm and 7% located between 60 and 90 cm. Some residual effect of the moisture treatments on root biomass was observed, with drought increasing root biomass at the 0 to 15 cm ($P = 0.04$) and 15 to 30 cm ($P = 0.02$) depths (data not shown).

DISCUSSION

There was little or no difference between the normal and wet treatments in terms of biomass production, leaf water relations, nutritional value, and root growth, suggesting that average rainfall for central Pennsylvania is sufficient to maximize forage production when moisture is evenly distributed throughout the growing season. Our simulation of drought levels that could be expected to occur about 1 in 10 yr resulted in an average 22% reduction in forage production compared with normal rainfall years. As would be expected, the negative

Table 6. Forage quality (CP, crude protein; NDF, neutral detergent fiber; IVTD, in vitro true digestibility) of two-species (Mixtures A and B) and five-species (Mixtures C and D) mixtures. Mixtures included the following species: (A) Kentucky bluegrass/white clover, (B) orchardgrass/red clover, (C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/chicory, and (D) Kentucky bluegrass/perennial ryegrass/tall fescue/red clover/narrow-leaf plantain. Data are averaged across years, harvest dates, and stubble heights. LSD (0.05) values are for mixture by water interactions for each forage quality parameter.

	CP			NDF			IVTD		
Mixtures	Dry	Normal	Wet	Dry	Normal	Wet	Dry	Normal	Wet
	mg g^{-1} dry weight								
A	217	216	225	312	348	329	865	882	893
B	177	187	187	473	452	447	843	865	860
C	181	185	181	368	370	384	887	891	889
D	173	185	193	423	412	403	829	844	857
LSD (0.05)		7			19			10	

Table 7. Root distribution under two-species (Mixtures A and B) and five-species (Mixtures C and D) mixtures. Data were collected on 21 May 2002 following the final harvest for species composition determinations. Mixtures included the following species: (A) Kentucky bluegrass/white clover, (B) orchardgrass/red clover, (C) Kentucky bluegrass/perennial ryegrass/orchardgrass/white clover/chicory, and (D) Kentucky bluegrass/perennial ryegrass/tall fescue/red clover/narrow-leaf plantain. Numbers in parentheses represent the contribution of roots at a given depth to total root biomass.

Soil Depth	Mixture A	Mixture B	Mixture C	Mixture D
	Kg ha^{-1}			
0–15 cm	1380 (85%)	609 (71%)	602 (52%)	1790 (73%)
15–30 cm	125 (8%)	168 (20%)	73 (6%)	323 (13%)
30–60 cm	54 (3%)	70 (8%)	390 (34%)	224 (9%)
60–90 cm	4 (< 1%)	10 (1%)	86 (7%)	128 (5%)
Total†	1624b	857c	1150bc	2465a

† Total root biomass data followed by the same letter are not significantly different at $P = 0.05$.

effect of drought increased across time, having no effect on growth rate in May but reducing growth by 33% in July and 43% in September. Although Mixture B had greater yield in the normal moisture treatment than the predominant Kentucky bluegrass/white clover mixture typically found in northeastern USA pastures, and Mixture D had greater yield in the wet treatment, neither mixture significantly increased yield over the Kentucky bluegrass/white clover baseline under drought. Only Mixture C significantly outyielded Mixture A at all three moisture levels.

Mixture C was dominated by chicory, which contributed 55% of total mixture biomass when averaged across harvests and years, and by September 2001 had reached 71% of mixture biomass. There is some controversy as to whether the sampling effect, whereby increased productivity with greater biodiversity results from the increased probability of a mixture containing the most productive species of the entire species pool, constitutes a true benefit of biodiversity (Tilman, 1999) or is simply an artifact of the way experimental communities are assembled (Wardle, 1999). In this experiment, the superior performance of Mixture C was clearly related to the presence of chicory in the mixture. In the dry and normal treatments, the contribution of chicory alone to forage yield of Mixture C was equal to the total yield of Mixture A, and Mixture C yield was similar to reported yields of pure stands of chicory at a nearby site (Jung et al., 1996). However, the additional combined biomass of the other species in Mixture C also amounted to about 85 and 65% of the total yield of Mixture A in the dry and normal treatments, respectively, suggesting that the improved performance of Mixture C was not solely because of the biomass produced by chicory. Relative growth rates of white clover and orchardgrass were also greater in Mixture C compared with their growth rates in simple mixtures.

Mixture C not only had the greatest forage yield across moisture treatments but also had more water remaining in the top 30 cm of the soil profile at the end of the experiment than any other mixture. It is possible that the deep root system of chicory allowed it to extract the bulk of its water from deeper in the soil profile, leaving more water available near the soil surface for use by other species in the mixture. Berendse (1982) suggested that the association of deep and shallow rooted species in mixture could cause greater nutrient extraction from deeper soil layers by the deep-rooted species than would normally be observed in monoculture. The same should also apply to moisture uptake, resulting in increased extraction from deep layers by chicory and a concomitant increase in moisture availability to neighboring species at shallow depths.

As another alternative, through the process of hydraulic lift, chicory may have redistributed water from relatively moist, deep soil layers to layers near the surface where it would then be available for uptake by other species (Richards and Caldwell, 1987; Caldwell et al., 1998). Dawson (1993) demonstrated that species differences existed in the ability of plants to use water supplied by hydraulic lift. Rhizomatous or stoloniferous perennials used the highest proportion of hydraulically lifted water, maintained higher LWPs and stomatal conductance, and showed greater aboveground growth than other species which used little or none of the water provided by hydraulic lift. In our experiment, only the stoloniferous species, white clover, showed improved leaf water relations in the complex mixture containing a deep-rooted species, although growth rates of orchardgrass were also improved. We do not have the necessary data to evaluate the relative importance of hydraulic lift vs. niche separation (or some other mechanism) as the cause for observed differences in soil water availability. Experiments are currently underway to further investigate this phenomenon.

Interestingly, the other five-species mixture (Mixture D), which essentially lacked a deep-rooted species because of the poor establishment and survival of plantain, had the least amount of water remaining in the upper soil profile in September 2001. Still, red clover growing in Mixture D had greater relative growth rates than red clover growing in the simple mixture (Mixture B) in all three moisture treatments and Mixture D yielded more than Mixture A in the wet treatment. It appears that in this case, species in the complex mixture were able to more fully utilize the moisture in the upper soil profile than species in the simple mixtures. This was consistent with the principles of niche utilization outlined by Tilman (1999).

Although the presence of chicory had a positive effect on forage yield and drought resistance, there is some concern in the literature about its long-term persistence (Hume et al., 1995; Li et al., 1997a). In our experiment, chicory increased throughout the experiment from 31% of harvested biomass in May 2000 to 46% in May 2001 and 49% in May 2002. The proportion of chicory in the mixture also increased during the growing season in each year. Belesky et al. (2000) found that chicory persistence in the second year of a clipping experiment was greatest with zero nitrogen inputs whereas chicory had nearly disappeared from the mixture with annual applications of 480 kg N ha^{-1}. We did not apply any nitrogen fertilizer to our plots but instead relied on nitrogen input from N_2 fixation by the legume component of the mixtures. Thus, it appears that chicory persistence might be improved under low fertility regimes or where N is provided by nitrogen-fixing legumes rather than by mineral fertilization. In addition, Li et al. (1997b) suggested that avoidance of grazing in late autumn would reduce winter injury and improve chicory persistence. In our experiment, the final clipping usually occurred around mid-September, which was several weeks before potentially damaging cold temperatures occurred and chicory winter survival was high (Skinner and Gustine, 2002). Many producers desire to extend grazing as late into the fall as possible. Doing so could reduce chicory persistence.

Red clover is another species with limited persistence. In the simple mixture, the proportion of red clover decreased from 43% in May 2000 to 23% in May 2002. Red clover was an important component of Mixture D and remained relatively constant across time, contributing 35% of harvest biomass in May 2000, 50% in May 2001, and 38% in May 2002. The other predominant species in Mixture D at the end of the experiment was tall fescue, which had increased from 3% of harvested biomass in May 2000 to 49% in May 2002. Thus, after 2 yr, Mixture D had essentially become a tall fescue/red clover simple mixture. Tall fescue is considered to be highly competitive with legumes including red clover (Fales et al., 1996). Studies in Northern Ireland found that tall fescue/red clover mixtures could maintain peak productivity for 4 to 6 yr, and when no fertilizer N was applied, red clover still contributed 36% of total biomass 8 yr after sowing (McBratney, 1981, 1984, 1987). Red clover was also the dominant component of a tall fescue-red clover mixture for all 3 yr of a of study in the southeastern USA (Hoveland et al., 1999) where heat, drought, pests, and warm-season grass competition make legume persistence difficult (Hoveland, 1989).

With the exception of Mixture A, mixture identity did not greatly affect nutritional value of the harvested forage. Differences among mixtures probably resulted from differences in botanical composition. In particular, the high CP and low NDF concentrations in Mixture A probably resulted from the high white clover content of the mixture. Mixture C, which had the highest IVTD, was dominated by chicory, which has relatively high nutritive value (Sanderson et al., 2003) and has been shown to enhance in vitro digestion kinetics when grown in mixtures with orchardgrass (Belesky et al., 1999). Digestibility decreased with drought stress in all mixtures except Mixture C. Differences in tissue age may have accounted for the reduced IVTD under drought. Because growth rates were reduced by drought stress, droughted plots for Mixtures A, B, and D took longer to reach the desired cutting height and plant tissues were, therefore, older when harvested than in the normal and wet treatments. In Mixture C, however, the dry treatment did not reduce the number of harvests or increase plant tissue age at harvest.

CONCLUSIONS

Even though all the alternative mixtures improved forage yield compared with the standard Kentucky bluegrass/white clover mixture (Mixture A) under certain environments, only the complex mixture containing chicory, white clover, orchardgrass, Kentucky bluegrass, and perennial ryegrass (Mixture C) was able to do so under all moisture conditions. Improved performance was due both to the presence in the mixture of the highly productive and drought-resistant species, chicory, and to the improved water relations and relative growth rates of white clover and orchardgrass. This suggests that both the sampling effect and facilitative interactions were operational in this system. Comparisons between the two complex mixtures suggests that including the additional functional attribute of a deep-rooted species in Mixture C was more important than species richness per se for improving forage yield and stability in these mixtures.

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